

Symbiosis as a Mechanism of Evolution: Status of Cell Symbiosis Theory*

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Abstract

Several theories for the origin of eukaryotic (nucleated) cells from prokaryotic (bacterial) ancestors have been published: the progenote, the direct filiation and the serial endosymbiotic theory (SET). Compelling evidence for two aspects of the SET is now available suggesting that both mitochondria and plastids originated by symbioses with a third type of microbe, probably a *Thermoplasma*-like archaebacterium ancestral to the nucleocytoplasm. We conclude that not enough information is available to negate or substantiate another SET hypothesis: that the undulipodia (cilia, eukaryotic flagella) evolved from spirochetes. Recognizing the power of symbiosis to recombine in single individual semes from widely differing partners, we develop the idea that symbiosis has been important in the origin of species and higher taxa. The abrupt origin of novel life forms through the formation of stable symbioses is consistent with certain patterns of evolution (e.g. punctuated equilibria) described by some paleontologists.

Keywords: cell evolution, cilia, endosymbiosis, flagella, macroevolution, microbial evolution, microtubules, mitochondria, plastids, punctuated equilibrium, semes, serial endosymbiotic theory, spirochetes, undulipodia

1. Introduction

Three alternative models of the origins of eukaryotes were presented in the geological treatise by Smith (1981) as if they merited equal treatment.

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Yet modern biological techniques, especially those of nucleic acid and protein sequencing, permit one of them to be clearly distinguished. The symbiotic theory of the origin of eukaryotic organelles best accounts for a vast amount of biological literature (Gray, 1983; Margulis, 1981).

The serial endosymbiotic theory (Taylor, 1974; Margulis, 1981) in its most extreme form states that three classes of eukaryotic cell organelles (mitochondria, plastids and undulipodia) originated as free-living bacteria (aerobic respirers, oxygenic phototrophic bacteria and spirochetes, respectively) in association with microbes that became the nucleocytoplasm (*Thermoplasma*-like archaeobacterial hosts). Molecular biological information, primarily derived from ribosomal RNA nucleotide sequencing studies leads to the conclusion that the symbiotic origin theory for both mitochondria and plastids has been proven (Gray, 1983). The probability of an ancestral archaeobacterial-*Thermoplasma*-like host for the nucleocytoplasm has been rendered more plausible by discoveries by Dennis Searcy and his colleagues (Searcy and DeLange, 1980; Searcy and Stein, 1980; Searcy et al., 1981) and Carl Woese and his colleagues (Woese et al., 1978). The most equivocal postulate of the symbiotic theory: the origin of undulipodia (cilia and other organelles of motility that develop from kinetosomes; Margulis, 1981, Margulis and Sagan, 1986) is under investigation now. The status of these postulates, as well as their implications for the fossil record, is briefly summarized here.

2. Alternative Theories

A consensus has been reached in the biological community: the largest discontinuity in the biological works is between the prokaryotes (organisms lacking nuclei) and eukaryotes (organisms composed of cells with nuclei). The bacteria, all members of the Monera kingdom, include actinobacteria, myxobacteria and cyanobacteria (formerly known as blue-green algae or cyanophytes). The eukaryotes include members of four kingdoms: Protoctista (protoctists, the eukaryotic microorganisms and their multicellular descendants); Fungi (haploid or dikaryotic conjugating heterotrophic organisms that develop mycelia from spores and that lack both undulipodia and embryos at all states of their development); Animalia (diploid organisms that develop from blastula embryos) and Plantae (haplodiploid organisms that develop from nonblastular embryos supported by sterile tissue). For a detailed and illustrated description of members of the five kingdoms see Margulis and Schwartz, 1982.

A long list of characteristics distinguish prokaryotes from eukaryotes, the only defining one of which is the nuclear membrane. All theories that connect eukaryotic origins to prokaryotic evolution fall into one of two categories:

exogenous (symbiotic theories) or endogenous (autogenous or differentiation theories). There is agreement amongst all serious authors that the smaller, more resistant, less morphologically complex and more metabolically diverse prokaryotes preceded eukaryotes in the history of life on Earth. Furthermore, because of the detailed biochemical similarities of all life on Earth, there is general agreement that eukaryotes are related to prokaryotes by descent. The issue of debate, however, concerns the mechanisms of evolution by which the eukaryotes evolved from the prokaryotes. Of the three theories presented as mutually exclusive alternatives in the geological encyclopedia of Smith (1981), two (the "progenote" and "serial endosymbiosis" theories) recognize symbiosis as a mechanism for the origin of organelles. The third, the "developmental theory" (which is the same as the "autogenous theory," the "direct filiation theory," the "differentiation theory" or the "classical theory") and its ramifications have been discussed in detail by Taylor (1976). Taylor made explicit the difficulties with the developmental theory. However, the data revised by Gray (1983) have led to the abandonment of all versions of the development theory in favor of a symbiotic origin of mitochondria and plastids. The progenote theory resembles the symbiotic theory in that it accepts bacterial ancestry for both plastids and mitochondria. Indeed there is good evidence for the multiple origin (polyphyly) of both plastids and mitochondria (Raven, 1970; Stewart and Mattox, 1984; Fox, 1985).

However, the progenote theory "suggests that eukaryotes did not arise from prokaryotes, but that both derived from a common ancestor, the progenote, some 3500 million years ago" (Smith, 1981). The progenote, "much simpler than the simplest present-day cells" is considered by Woese (1981) to be the universal common ancestor of all life. No extant co-descendant of the progenote survives. Nor is any experimental evidence for the existence of progenotes available.

In favor of the symbiotic theory, Searcy and his colleagues have amassed experimental evidence supporting the idea that the original host that became the eukaryotic nucleoplasm was a *Thermoplasma*-like archaebacterium (Searcy and DeLange, 1980; Searcy et al., 1981). The evidence that the ancestor to the nucleocytoplasm of eukaryotes was indeed a prokaryote includes the presence of histone-like protein, called HT-A, bound to *Thermoplasma*'s DNA, *Thermoplasma*'s microaerophilic respiratory metabolism and glycolysis, and its status as an archaebacterium (Fox et al., 1980). The report of introns in the ribosomal genes of archaebacteria reinforces Searcy's concept that an archaebacterium like *Thermoplasma* was ancestral to the nucleocytoplasm of eukaryotes.

Thus, even though there is now some agreement about the multiple symbiotic origin of the two classes of organelles of eukaryotes, mitochondria and plastids, there are a number of "genesis" issues that remain unresolved. For example, what was the "progenote," *Thermoplasma* or other ancestral host that became the nucleocytoplasm? Is the nucleocytoplasm polyphyletic? How did the nucleus itself as an organelle originate? Furthermore, there is no consensus concerning the origin of mitosis and meiotic sex, of undulipodia, of the larger eukaryotic ribosomes, of the widespread distribution of repeated and intervening sequences in eukaryotic DNA's and other aspects of eukaryotic genetic processing. The following comments, presented within the confines of the scientific "thought-style" (Fleck, 1979) of the serial endosymbiotic theory (the SET), summarize the status of these various unresolved aspects of the SET. Although possible explanations are listed here, they are certainly only provisional at this point.

3. Status of the Serial Endosymbiotic Theory (SET)

The usefulness of the serial endosymbiotic theory is attested by its great explanatory power, which has generated productive scientific studies. The SET has begun to unite formerly disparate subfields of biology. The best example is probably the unification of organellar genetics and the physiology of symbioses into the new field of "Endocytobiology" (Schwemmler and Schenk, 1980, 1983). Beginning in 1978 as a discussion meeting at the Royal Society (Richmond and Smith, 1979) a significant advance was made when the New York Academy of Sciences meeting was held in 1980, bringing together organellar geneticists with specialists in symbiosis (Fredrick, 1981). A second New York Academy meeting on this subject will be held in June 1986, this time in connection with the new Society for Endocytobiology. This brief paper cannot begin to review the burgeoning literature; it can only indicate some major aspects of cell symbiosis theory. The issues and their status are listed here.

Mitochondria and plastids from bacteria

The origin of mitochondria and plastids from free-living bacteria ("eubacteria" rather than "archaeobacteria") can be considered established (Gray, 1983). The most likely candidates for co-descendants and hosts include those listed in Table 1. The phototrophic bacteria most likely to resemble the ancestors of the plastids are listed in Table 2. The relationship between organelles and selected extant bacteria is diagrammed in Fig. 1.

Table 1. Polyphyletic origin of plastids (photosynthetic organelles of eukaryotes).

Groups of photosynthetic organisms	Type of plastids	Possible extant photosynthetic prokaryotic co-descendants (phototrophic bacteria)	Possible co-descendant hosts with common heterotrophic ancestors (protists)
Plants (K)	chloroplasts	<i>Prochloron</i>	mastigotes
Chlorophytes (P)	chloroplasts	<i>Prochloron</i>	mastigotes
Euglenids (P)	chloroplasts	<i>Prochloron</i>	kinetoplastids
Rhodophytes (P)	rhodoplasts	<i>Synechococcus</i> , or other coccoid cyanobacteria	<i>Taphrina</i> -like fungi
Cryptophytes (P)	chrysoplasts	<i>Heliobacterium</i> ? ¹	cryptomonads
Chrysophytes (P)	chrysoplasts	<i>Heliobacterium</i> ? ¹	bodos, kinetoplastids, other mastigotes
Phaeophytes (P)	chrysoplasts	<i>Heliobacterium</i> ? ¹	heterokont mastigotes
Dinoflagellates (P)	chrysoplasts	<i>Heliobacterium</i> ? ¹	heterotrophic dinomastigotes

K - Kingdom, P - Phylum, ¹See Margulis and Obar, 1985

Table 2. Organelles and their possible co-descendant bacteria

Organelles	Possible co-descendants ¹
<i>Ubiquitous or nearly ubiquitous organelles</i>	
nucleocytoplasm	<i>Thermoplasma</i> , other archaeobacteria
mitochondria	<i>Bdellovibrio</i> , <i>Daptobacter</i> <i>Paracoccus</i> <i>Rhodopseudomonas</i>
plastids	See Table 1
undulipodia (cilia)	<i>Spirochaeta</i>
<i>Organelles of limited distribution</i>	
alpha (of <i>Paramecium</i>)	<i>Cytophaga</i>
kappa (of <i>Paramecium</i>)	<i>Caenobacter</i> -like gram negative free-living bacteria
pi (of <i>Paramecium</i>)	<i>Pseudocaenobacter</i> -like gram negative free-living bacteria
hydrogenosomes	<i>Clostridium</i>
cyanelles	<i>Synechococcus</i>
luminescent bodies (teleost fish)	<i>Photobacterium</i> , <i>Beneckea</i> (<i>Vibrio</i>)

¹ All prokaryotes, members of Kingdom Monera

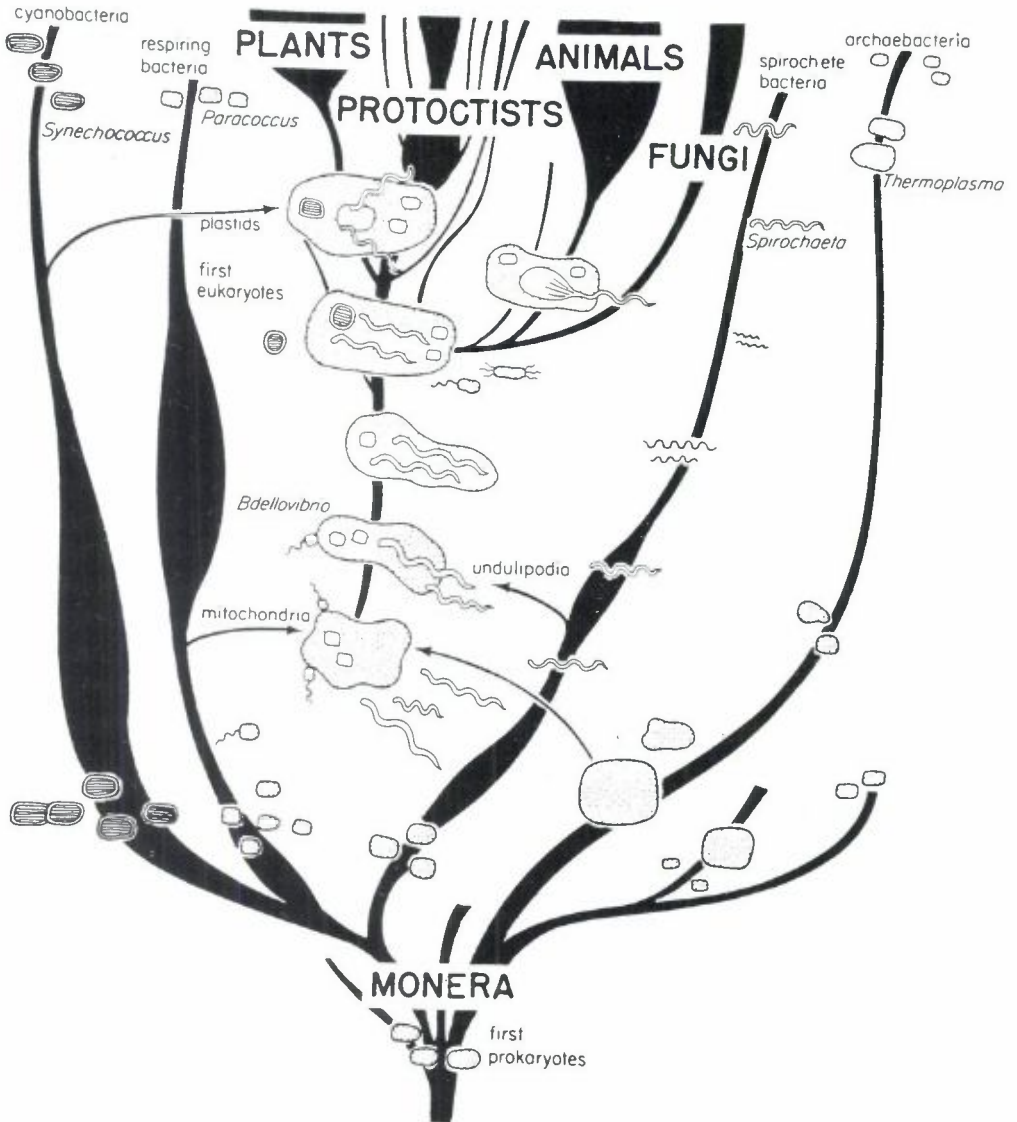


Figure 1. Organelles and their possible bacterial ancestors: origin of eukaryotes from prokaryotic communities (From Margulis and Sagan, 1986).

The polyphyly of mitochondria

The polyphyly of mitochondria is very likely; the tubular vs. flattened cristae in different lineages is quite suggestive that mitochondria evolved polyphyletically (Stewart and Mattox, 1984). New data have revealed an enormous variation in mitochondrial DNA sequences (Fox, 1985). Whether the variation is due to original polyphyly or diversification subsequent to the establishment of the symbioses (or both, as is most likely) has not been established. Several kinds of predatory bacteria are now known: the epibiotic *Vampirococcus* and *Vampirovibrio*, the endobiotic *Daptobacter* and the periplasmic *Bdellovibrio* (Guerrero et al., 1986; Fig. 2). There is no doubt then that certain prokaryotes penetrate other kinds of prokaryotes forming endocytobiotic relationships. These kinds of associations can be considered preadaptations in the origins of intracellular organelles.

The origin of the nuclear membrane

Arguments that the nuclear membrane and synthesis of its steroid components involves metabolic products of at least two originally different genomes (host and mitochondria) have been presented elsewhere (Margulis, 1981; Margulis and Sagan, 1986). The origin of the entire endomembrane system (e.g., nuclear membrane, endoplasmic reticulum, Golgi apparatus, outer membranes of mitochondria and plastids, vacuolar membranes, steroid-containing plasma membrane and so forth), is thought to have required the interaction of at least these two genomic systems (host and mitochondrial).

Origin of undulipodia (cilia and other 9+2 organelles)

Both endogenous (Pickett-Heaps, 1974; Wheatley, 1982; Cavalier-Smith, 1982; Dustin, 1983) and exogenous origins (Margulis, 1981; Margulis and Sagan, 1984) for the centriole-kinetosome-undulipodial complex (kinetids) have been presented. The discovery of tubulin-like proteins in a free-living spirochete (Fracek, 1984; Fracek and Stolz, 1985; Obar, 1985) increases the probability of the exogenous origin. The usefulness of the exogenous theory has also been demonstrated for the analysis of ciliate cortical development and ciliate speciation as well as for understanding the origin of meiotic sex (Margulis and Sagan, 1986).

Kinetids and their structure provide outstanding conserved characters on which to base protist classification and phylogeny (Barr, 1981; Stewart and Mattox, 1984). The observation of the usefulness of kinetids in the interpretation of protist phylogeny can be derived from the concept of the

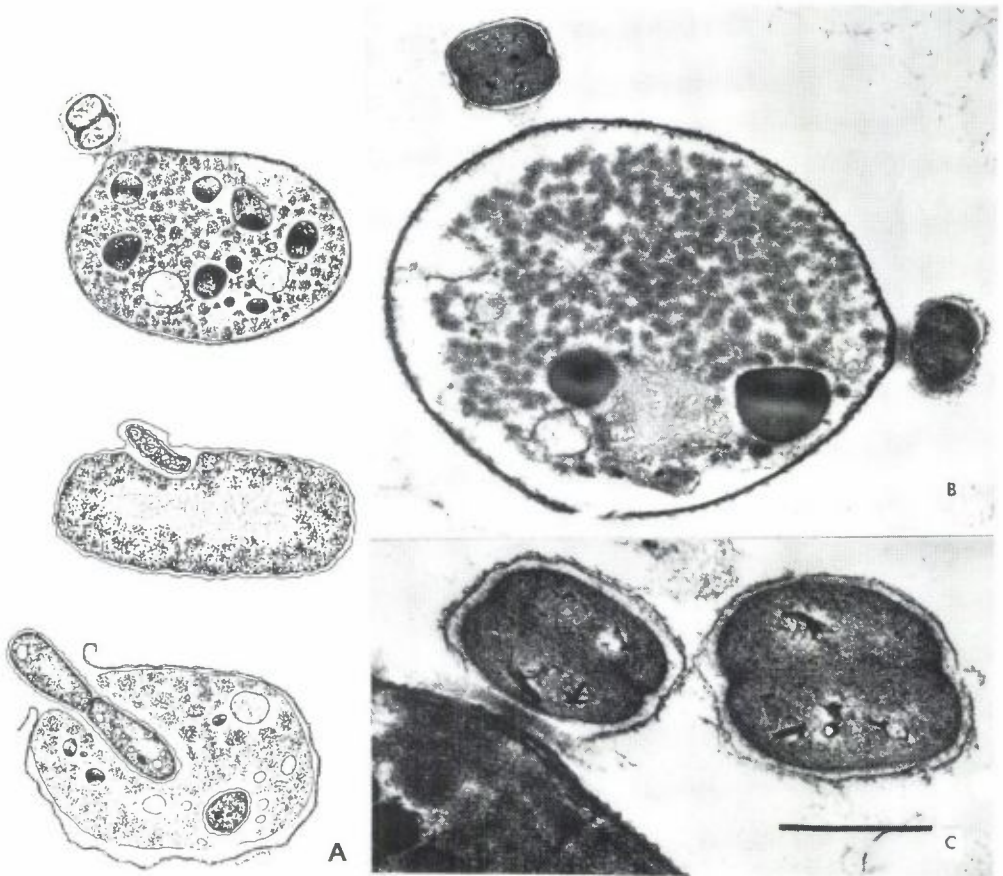


Figure 2. Predatory bacteria: A. Top *Vampirococcus*, center *Bdellovibrio* and bottom *Daptobacter*, drawing by Christie Lyons. B. Two *Vampirococcus*, in division, destroying the photosynthetic membranes of the *Chromatium* prey to which they are attached. C. Specialized cell wall attachment organelle of *Vampirococcus* (which is dividing) while attached to *Chromatium minus*. Description of this *Vampirococcus* and of *Daptobacter*, the predatory prokaryote which divides inside the cytoplasm of its prey, is in press, Guerrero, et al. 1986. Scale bar equal to 0.5 micrometers.

spirochetal origin of undulipodia and the recognition of the relationship between the kinetid and mitosis (Margulis and Sagan, 1986). Definitive comparisons of kinetosomal RNA sequences with DNA from spirochetes that synthesize tubulin-like protein are crucial for the resolution of this problem. An abundance of amino acid sequence data from proteins, for example, the tubulin-like protein and other microtubule-associated proteins linking undulipodiated motility systems more to spirochetes than to other prokaryotes will also be required before the theory can be considered proven.

Microtubules in spirochetes and other prokaryotes

Various kinds of microtubules, in the sense of small, hollow-walled structures, have been observed in thin section electron microscopy in about twenty kinds of prokaryotes including several species of cyanobacteria. These include *Anabaena minutissima*, *A. cylindrica*, *Nostoc sphaericum*, and *Nostoc* sp. culture #756 from the culture collection of algae at the University of Texas, Austin (Jensen, 1984), as well as a nitrogen-fixing oxygen-respiring gram negative motile rod bacterium *Azotobacter vinlandii*, and several genera of spirochetes such as *Treponema*, *Leptospira*, *Hollandina* and *Pillotina*. The size of these tubules varies from fewer than 7 to about 24nm. Based on antigenic reaction to polyclonal antibodies made against authentic brain or mitotic spindle tubulins, tubulin-like protein has been found in *Azotobacter* by Adams (1983; also see Adams and Kelley, 1984) and in spirochetes by Fracek (1984), Fracek and Stolz (1985), and Obar (1985). An antitubulin antibody reaction has been detected in both whole cells and extracted proteins of *Spirochaeta bajacaliforniensis* (Bermudes and Margulis, 1985). This new marine spirochete, although it lacks cytoplasmic microtubules, seems to have fibers in its protoplasmic cylinder (Fracek and Stolz, 1985). The antigenically active protein can be purified by the standard warm-cold cycling technique for brain tubulin purification. The antitubulin reaction in *S. bajacaliforniensis* is to an acidic protein of MW 67,000 daltons called S1. This tubulin-like protein has been purified and its peptide digests studied by Obar (1985). The determination of enough of the S1 amino acid sequence to establish whether or not tubulin and S1 are homologous has not yet been accomplished.

The sequence of acquisition of organelles

Some protists apparently never acquired mitochondria, e.g., *Pelomyxa*, *Entamoeba* (Fahey et al., 1984) and maybe even parabasalids such as *Trichonympha* (Roberts et al., 1981). Because all photosynthetic eukaryotes that contain plastids also contain mitochondria, plastids most likely were acquired by organisms that already had acquired mitochondria. The acquisition of undulipodia may have preceded the acquisition of mitochondria (as pointed out by Schwemmler, 1984 and Kunicki-Goldfinger, 1980). J.L. Griffin (1979) has observed immotile undulipodia on the surface of *Pelomyxa*, an organism that lacks mitochondria, chromosomes, mitosis and meiotic sexuality (Whatley and Chapman-Andresen, 1986). These observations support the idea that the acquisition of undulipodia preceded the origin of mitochondria.

Large ribosomes

The sizes of cytoplasmic ribosomes and their complexity varies a good deal in eukaryotes, which in general have the larger 80S ribosomes rather than the small 70S ribosomes of prokaryotes. A consequence of the spirochete postulate of the SET is the concept that eukaryotic ribosomes are recombinant structures of dual origin: host (presumably archaebacteria) and undulipodial ancestor (presumably spirochete). This idea is verifiable by molecular biological data. The sequence of nucleotide bases and amino acids in ribosomal RNA's and proteins of eukaryotes should show more homology to, for example, those of the ribosomal RNA's and proteins of *Thermoplasma* and *Spirochaeta bajacaliforniensis* than to arbitrarily chosen prokaryotes.

Genome organization

Genomic organization in eukaryotes, even microorganisms such as yeast and ciliates, is far more complex than that of prokaryotes. A postulate of the SET is that eukaryotic genome did not just differentiate from the ancestral host prokaryotic genome but that it is of at least dual origin: host and spirochete and, therefore, the genomic system itself is recombinant in all of its aspects. Indeed, since in most eukaryotes genes have been transferred from the mitochondria to the nuclei of the host, the eukaryotic genome derives from at least three heterologous genomes: host, spirochete and mitochondria. Much molecular biological literature provides evidence that originally mitochondrial genes have been transferred to the nucleus (Obar and Green, 1985). On this view the nuclear genome originates from at least three heterologous genomes. For example, the DNA of heterochromatic regions of chromosomes, especially kinetochoric DNA, is thought to be of spirochetel

origin (Margulis and Sagan, 1986). Structures like the telomeres of the chromosomes of the ciliate *Oxytricha* with nucleosomes phased inward showing complexes of about 100 base pairs terminating in CCCC AAAAGGGG TTTT (Gottschling and Cech, 1984), are considered to be emergent properties of genomes originating from two different nonhomologous bacterial ancestors. Many details require analysis but such an analysis is not possible if the chimeric nature of the eukaryotic cell is not acknowledged.

Animals and plants

The concept that the Archaen Aeon was the "age of anaerobic bacteria," the Proterozoic Aeon the "age of aerobic bacteria" and the Phanerozoic Aeon the "age of prototists" (which began as expanding bacterial mergers) is consistent with both the fossil record and modern evolutionary biology. Plants, animals and fungi can be thought of as such prodigious lineages of prototists that they now deserve kingdom status.

Systematics and taxonomy of the microorganisms

The recognition of the monogenomic nature of prokaryotes and the polygenomic nature of eukaryotes has immeasurably clarified classification schemes for the organisms of the microcosm: bacteria and prototists (Margulis and Schwartz, 1982; Fig. 3; Corliss, 1984).

4. Implications for the Fossil Record

There is direct evidence that the eukaryotic level of organization was reached and preserved in the fossil record during the Proterozoic Aeon. Robust, relatively complex and relatively abundant cyst-like structures (acritarchs) have been found in macerations of shales from several fossil localities (Vidal, 1984; Vidal and Knoll, 1983).

5. Symbiosis as a Mechanism of Evolution

Various biological conditions and phenomena, coupled with the bacterial nature of mitochondria and the responses of organisms to microbic invasions, leads the author to conclude that *symbionticism is the fundamental factor in the origin of species.*

I.E. Wallin, 1927

Speciation has been traditionally considered to be a result of environmental selection on a sequence of randomly acquired, small genetic changes. We suggest speciation often occurs by symbiosis, a discontinuous mechanism of

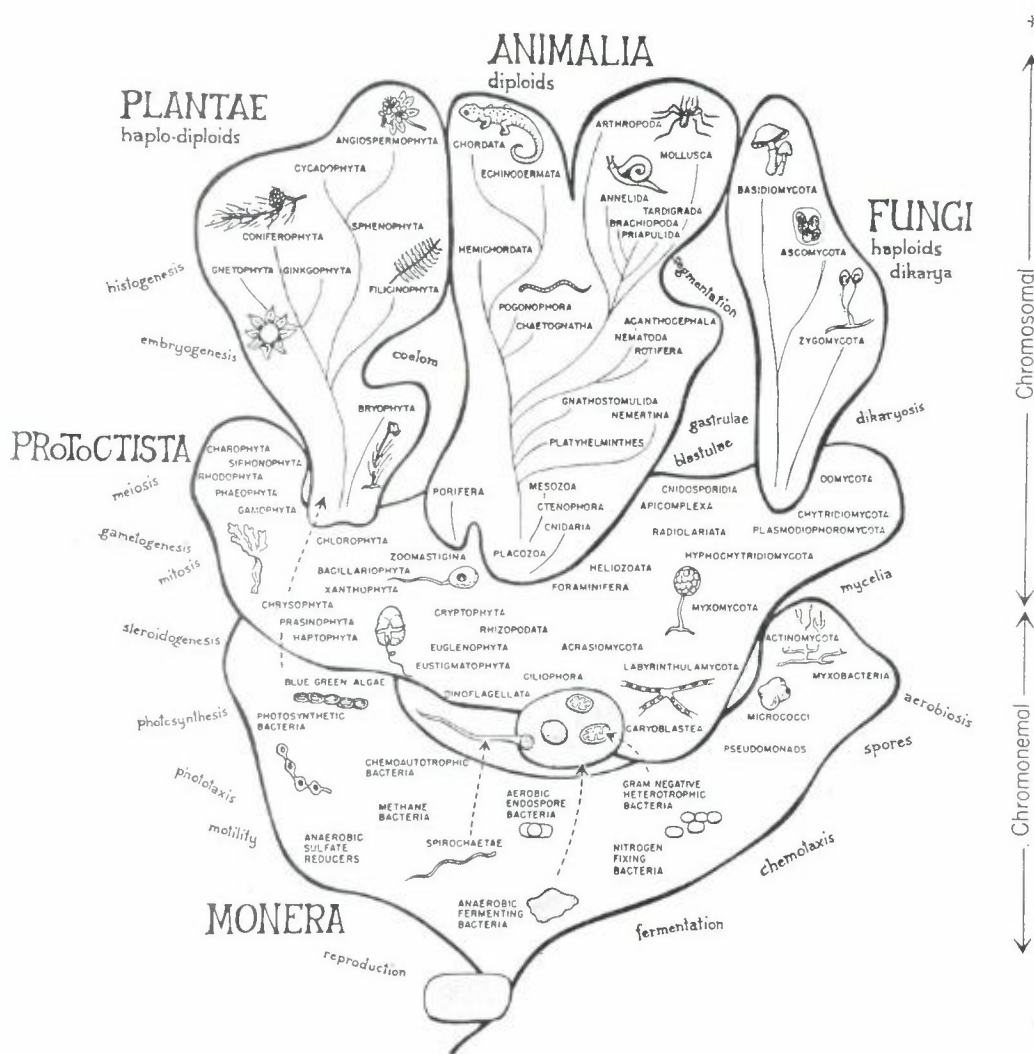


Figure 3. Evolutionary history and possible relationships between the highest taxa, Whittaker's five kingdoms of life, slightly modified. MONERA (bacteria, all prokaryotes); PROTOCTISTA (Eukaryotic microorganisms beginning as microbial communities and their descendants exclusive of animals, fungi and plants); ANIMALS (diploids developing from blastulas); FUNGI (haploids or dikaryons developing from spores); PLANTS (plastid-containing haplodiploids developing from embryos supported by sterile tissue). From Margulis, 1981; (see Margulis and Schwartz, 1982 for details of members of groups). * Type of DNA organization: chromonomal of the genophore of prokaryotes and chromosomal of the histone-coated DNA of eukaryotes.

large steps. In symbiotic speciation an entire functional genome and the associated protein synthesis system of one organism is acquired by another organism. We suspect that symbiosis, in bringing together divergent genomes, has created some of the major cellular innovations responsible for the origin of some higher taxa.

Goldschmidt (1933, 1940) argued that higher taxa may evolve through major genetic reconstruction, the product of which he dubbed the "hopeful monster." This mode of evolutionary change has been termed macrogenesis. Claiming a lack of supporting evidence for macrogenesis, Mayr (1963) argued for such a high probability of failure that only a "hopeless monster" would be created. In a sense, symbiotic acquisition provides Goldschmidtian macroevolutionary mechanisms: the symbiotic complex represents major genetic reconstruction with synergistic capabilities unavailable to its unassociated components. We believe that symbioses rather than accumulation of random mutations may be a major mechanism in large-scale evolutionary change underlying the evolutionary appearance of some higher taxa. We suggest that qualitative differences in semes (inherited characters, the product of natural selection, functioning together) outlined below underlie the appearance of higher taxa (kingdoms, phyla, classes) and can be distinguished from semes that underlie lower taxa (genera, species, varieties). We hypothesize that the role of symbiosis has been especially important in evolution at higher taxonomic levels.

1. Higher taxa share highly conserved features

Kingdom and phyla in any systematic hierarchy, are not simply headings in a system of classification but represent groups of organisms sharing common semes (e.g., animals as eukaryotic organisms that develop from a blastula (Margulis and Schwartz, 1982)). Since the defining features of higher taxa are universally present in the members of the group, by definition they are highly conserved. Examples of universal semes include the nuclear membrane as the defining feature of the eukaryote superkingdom and a multicellular embryo supported by sterile, plastid containing tissue as the defining feature in the plant kingdom.

2. Highly conserved semes are probably impossible or surely disadvantageous to alter

A seme may be conserved because it is essential, difficult to change (requiring many mutations), or because it confers such a great selective advantage that without it organisms face death. For example, plastids in chlorophytes and angiosperms are not known to be lost. Even etiolated and parasitic plants have some form of plastid such as proplastids, etioplasts or leucoplasts.

3. The origin of higher taxa initially involves dramatic changes in or the appearance of highly conserved semes (e.g., neosemes, Hansen, 1977).

That higher taxa have highly conserved semes that are life-threatening to lose or alter suggests the origin of higher taxa initially involves dramatic changes in or appearance of such highly conserved semes. The origin of higher taxa has been generally considered to occur at the species level and to result from subsequent speciation (ramification). However, the ancestral species in a lineage must contain some neoseme (newly-appearing seme) that is conserved such that all descendant species are united by sharing it. Semes such as nuclear membranes and plastids are highly conserved. Therefore, we infer that the appearance of these semes correlates with the appearance of, or change in, taxa. Although these changes can only be determined in retrospect, to understand the origin of higher taxa we must understand the origin of neosemes.

4. The origin of lower taxa involves less highly conserved semes

Semes defining lower taxa are not highly conserved; they tend to change many times. For example, flowers of angiosperms change form (family level evolution) while the presence of the flower is constant (conserved phylum level feature).

5. Symbiotic events correlate with the origin of both higher and lower taxa (conserved and nonconserved semes)

To understand the origin of new taxa we must understand the appearance of neosemes. Acquisition of symbionts correlates with the appearance of neosemes and the origin of new taxa in some cases.

Several higher taxonomic features correlate with the acquisition of symbionts. The origin of eukaryotes (superkingdom) correlated with the acquisition of symbiotically-derived organelles (Margulis, 1970, 1981; Schwemmler, 1984). Thus, the most fundamental distinction in cell organization (prokaryote/eukaryote) and one of the most highly conserved semes, the nuclear membrane (a neoseme), probably originated as an emergent property of cell symbiosis. Several phyla (e.g., Rhodophytes, Phaeophytes, Chlorophytes) have conserved features (plastids and their accompanying photosynthetic pigments) that are also believed to represent symbiotically-derived organelles. In each case, plastids are a defining characteristic of these taxa. Indeed, the origin of land plants has been attributed to mycorrhizal associations with fungi (Pirozynski and Malloch, 1975); these symbioses are thought to have contributed to enhanced desiccation resistance and nutrient uptake.

Lower taxa also may appear because of the acquisition of stable symbionts. Termites may have speciated in ways correlated with changes in their microbial symbionts responsible for digestion. Without their endosymbionts, termites are unable to obtain essential nutrients from their diet. Thus it is unlikely that they could make changes in diet associated with speciation without a change in symbionts.

6. Higher taxa result from symbioses that unify organisms which display extremely different semes.

The great selective advantage in many symbiotic associations accrues from the extremely divergent capabilities that are brought together. The light harvesting and N_2 -fixing metabolism of cyanobacteria differs widely from the heterotrophic metabolism of the fungi with which some cyanobacteria form stable symbioses (lichens). Cryptomonads such as *Cyanophora paradoxa* appear to have recently engulfed and retained cyanobacteria which now exist as cyanelles. In cyanelles of *Cyanophora* (as in general with symbiotically derived organelles) the amount of genetic information has been reduced to only 10% that of their free-living cyanobacterial co-descendants (Herdman and Stanier, 1977). Apparently, loss of *redundant* genetic information (see Karakashian, 1963) and simultaneous retention of *divergent* genetic information is a property of the establishment of stable symbiont complexes. Symbiotic associations may form between organisms that are relatively similar in their metabolism (e.g., heterotrophic bacteria and heterotrophic protists as in the bacterial symbionts of *Amoeba* or *Paramecium*). They may also occur between organisms displaying vastly different lifestyles and metabolism (photoautotrophic algae and bivalve mollusks). It seems obvious that the latter types of associations between very different organisms have the most profound and immediate evolutionary consequences for the origins of higher taxa.

7. Symbiotic events may underlie recognized correlates of evolution such as "punctuated equilibrium"

Acquisition of symbionts may underlie some of the observation of "punctuated equilibrium," that is, discontinuity in lineages as observed in the fossil record (Eldredge and Gould, 1972; Gould and Eldredge, 1977). Symbiont acquisition may precipitate a rapid change in habitat and ecology within a lineage.

The rapid heritable changes due to the symbiotic association of *Amoeba proteus* and endosymbiotic bacteria has been summarized by Jeon (1983). The initial invasion of the bacteria, fatal to most of the amoebae, led to the

appearance of a few resistant members of the population. Ancestral amoebae were selected for tolerance to the pathogenic bacteria (Jeon, 1972; Jeon and Jeon, 1976). After fewer than 1000 amoebal generations, alterations in growth rate, temperature sensitivity, and nuclear compatibility (in experimental microsurgical nuclear transplants) occur and the amoebae become dependent on their former pathogenic bacteria for survival. Such patterns of sudden change are suggestive of punctuated equilibrium. Permanent genetic changes resulting from symbiotic association have been discussed elsewhere (Margulis, 1976, 1980, 1981).

The acquisition of symbionts appears to act as a mechanism that isolates a population from its nonsymbiotic ancestors. Symbiont acquisition may simultaneously change both habitat and ecology of the population in question. A behavioral shift (phototropism), due to the acquisition of a photosynthetic symbiont causing a change from heterotrophy to autotrophy, is clearly documented in the case of *Paramecium bursaria*. All *Paramecium* species, with the exception of *Paramecium bursaria* which harbor symbiotic *Chlorella*, are insensitive to visible light (Dryl, 1974). Acquisition of phototrophic symbionts tends to lead to a change in habitat (from dark to light), thus separating the symbiotic descendants from their nonsymbiotic ancestors. The details of population change generated by such "founder" individuals is not understood but we suggest that "founder effects" may occur not just as a result of isolation, but because of continuing integration of symbionts (Margulis, 1976, 1980, 1981).

6. Summary

Although the entire serial endosymbiotic theory has not been proven, much progress has been made and the nature of the remaining critical observations can now be identified. The symbiotic and multiple origin of eukaryotes was clearly recognized by K. Mereschkowsky (1905, 1910; Fig. 4) and by Wallin (1927). Both these biologists argued for symbiotic origin of plastids and mitochondria but they did not develop symbiosis as a mechanism of cell evolution for intracellular (including undulipodial) motility. Wallin, who also emphasized the importance of symbiosis as a mechanism of speciation and the role of intracellular symbiosis in embryological processes, was entirely ignored by "mainstream" biology (Mehos, 1983). It seems probable to us that Wallin was correct. His "symbiontism," the establishment of obligate associations between organisms of different species, is probably more important than the accumulation of single mutations, in the origins of major taxa of protocists and probably those of other eukaryotes. Furthermore, differential replication and metabolism of intracellular symbionts is likely, as Wallin surmised, to be

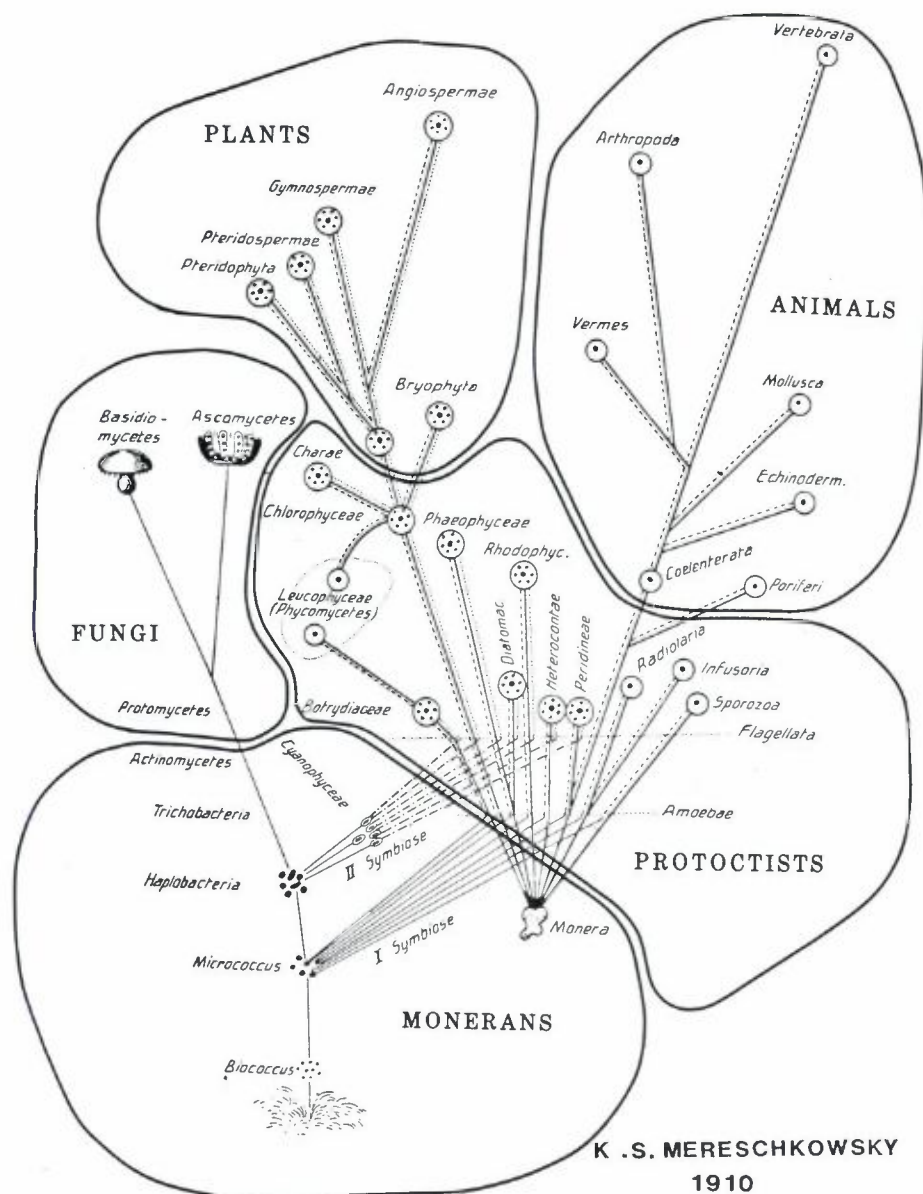


Figure 4. Mereschowsky's anastomosing phylogeny based on symbiosis with the current five kingdom superimposed. The major correction of Mereschowsky's work that needs to be made is the recognition of the bacterial nature of "actinomycetes" (actinobacteria); e.g., fungi, as eukaryotes, could not have evolved directly from these prokaryotes.

a fundamental mechanism of differentiation and embryological development in plants and animals (Margulis and Sagan, 1986).

There is little doubt that, regardless of the details, prokaryotes are single genomic systems and all eukaryotic cells are polygenomic ones. As implied by Mereschkowsky as early as 1910 (Fig. 4) the polygenomic nature of eukaryotes has serious ramifications for taxonomic schemes involving the highest taxa. To us it reinforces the imperative of Whittaker's (1969) five kingdoms (Fig. 3). Eukaryotic cells and the organisms that develop from them are, therefore, best thought of as *co-evolved bacterial communities*. The shift in symbionts, that is, in microbial community membership, are directly correlated with the appearance of new taxa in certain cases. Large eukaryotic organisms are entities that emerged as the bacterial symbiotic partnerships became tightly integrated during the late Late Proterozoic Aeon about 1,000 million years ago.

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